REMARKS

The claims have been amended to expedite prosecution. Claim 1 has been amended for clarity, but also to include an additional requirement formerly in claim 38 requiring an overall cationic charge, and perhaps more importantly, to limit the claim to those amounts of polynucleotide administration where the immune response is elicited.

The amendments to the remaining claims are simply to clarify claim wording and do not add new matter. Entry of the amendment is therefore respectfully requested.

It is understood that there is no requirement for the Examiner to enter the amendments at this stage of the prosecution. However, in view of the fact that the bulk of the amendments simply clarify the claim wording and, applicants believe, "clean up" the claims, no further searching or examining effort on the part of the Office is believed to be required. As discussed at the interview, the only amendment to claim 1 that changes its scope was already in claim 38 and the additional amendment to limit the claim to those amounts of polynucleotide administration where the immune response is elicited is merely intended to focus the claims on the valid unexpected results obtained with the invention compositions. A clean copy of the claims as proposed to be amended is included as Exhibit A to aid in the evaluation of these amendments.

The Invention

The invention resides in the discovery that encapsulating, into the intravesicular space of liposomes, expression systems for a peptide against which an immune response is desired yields superior results to methods taught in the prior art for administering such expression systems. These prior art methods include the naked DNA methods of Liu and Weiner and the complexation

methods taught by Felgner. All of the outstanding rejections are made over combinations of these documents with Kirby as further set forth below.

It is understood that the method of Kirby was used to prepare the compositions of the present invention. However, there is no suggestion in the art to use these methods for preparation of liposomes that contain desired expression systems, and further, using the method of Kirby so as to encapsulate the expression system in the intravesicular space results in compositions that yield unexpectedly favorable results.

The Rejections

All claims except claim 7 were rejected as assertedly obvious over Felgner, *et al.* (US 5,264,618) in view of Kirby, *et al.*, *Biotechnology* (1984) 2:979-984 and Weiner (US 5,593,972) or in the alternative over Felgner and Kirby in view of Liu, *et al.* (WO 95/24485). Applicants acknowledge the teachings of Kirby, *et al.* as describing a process which permits successful incorporation of nucleic acids into intravesicular space of liposomes. However, none of the remaining documents effectively teach that such incorporation is desirable, nor do they predict that compositions wherein such incorporation has occurred would be more effective than mere complexation with liposomes or more effective than simply naked DNA.

Arguments Regarding a *Prima Facie* Case

Applicants first present their arguments for the proposition that no *prima facie* case exists with respect to the claimed invention.

Felgner is characterized by the Office as teaching methods of inducing an immune response in an animal by delivering compositions comprising cationic liposomes "encapsulating" polynucleotides that encode immunogens.

However, Felgner actually teaches away from actual entrapment into the intravesicular space, because mere complexation of polynucleotides with lipid vesicles is taught. "Encapsulation" is not defined by Felgner as restricting the polynucleotide to the intravesicular space. The reference to "encapsulation" appears, as far as applicants can see, only in column 8 at lines 27-34. It is unclear what the terms "incorporating or encapsulating" in line 28 actually refer to – such terms are often used to describe mere association of active agents with liposomes.

As has been pointed out previously in the prosecution of this case, the only Felgner exemplified composition (Example 13) that includes polynucleotides clearly relates to complex formation, not incorporation into intravesicular space of liposomes. Taken as a whole, Felgner appears to suggest that the cationic lipids (that are described by Felgner as novel and inventive) are simply able to associate with polynucleotides (due to their negative charge) as an aid in cellular transfection. Any liposomes described by Felgner appear to be used as cell penetrating agents irrespective of the status of the polynucleotide or other biologically active agent.

In column 8, beginning at line 27, it is noted that the intracellular transport can be accomplished by incorporating or encapsulating the bioactive agent in the lipid vesicle prior to contacting the relevant cells, or the biologically active agent and the lipid vesicles can be simply administered together without prior association. That having the nucleic acid reside in the intravesicular space is irrelevant and not necessarily desirable is taught by Felgner in column 8,

lines 31-34 and 42-50, where apparently the biologically active agent and cationic lipid are administered separately.

A fair review of Felgner would not lead the reader to conclude that Felgner is suggesting the incorporation of an expression system for generating an immunogen into the intravesicular space, as opposed to a genus that includes any kind of association at all with cationic lipids, whether the cationic lipids are in the form of liposomes or not. Thus, the present invention might be considered a selection invention. To the extent that Felgner might be viewed as disclosing providing expression systems in the intravesicular space, as required by the present claims, it is an unspecifically described member of a genus that permits any kind of association of an expression system with cationic lipids.

Thus, Felgner fails to disclose the specific embodiment claimed in the present invention, whereby the expression system for the immunogen is contained in the intravesicular space *per se*, as opposed to a simple association with cationic lipids as carriers.

There is no reason that the reader of Felgner would apply the method of Kirby to ensure encapsulation of the expression system. According to the teachings of Felgner, all that is required is a simple association of such expression systems with a cationic lipid. Why go to the trouble of encapsulating?

The Office suggests that motivation might be found in attempting to protect the expression systems from nucleases, thus enhancing the stability of the active agent. However, the specification itself demonstrates that no significant protection is obtained to DNase in comparison to that afforded by mere complexation. Entrapment does apparently no better job that such complexing.

As stated on page 19 of the specification, at lines 19, *et seq.*, for DNA incorporated into liposomes the percentage not degraded by DNase was:

For neutral liposomes 45-72%;

For negatively charged liposomes 58-69%;

For cationic liposomes 68-86%.

While these numbers are slightly higher than for DNA adsorbed to the surface of cationic liposomes (41-58% not available for degradation), the differences are not in any way spectacular. The protection afforded by simple complexation appears comparable to, though slightly lower than, that for entrapment in any of the liposomes.

As noted in the recent decision in *KSR International Co. v. Teleflex, Inc.*, 82 USPQ2d 1385 (S. Ct. 2007), in order for a combination of documents to defeat patentability, the practitioner must have some reason to combine these teachings. While the strict teaching - suggestion - motivation (TSM) test was rejected by the Court, clearly the decision in *KSR* did not hold that the Office is permitted to use the teachings of the invention itself as a guide to picking out documents that disclose elements of the invention. As noted above, Felgner appears merely to teach a genus of associations between cationic lipids and expression systems for immunogens and fails to guide the reader to select those where the expression system is in the intravesicular space. Indeed, Felgner appears to teach away from this, and to teach that the manner of association is irrelevant to efficacy. To the extent Felgner suggests a particular embodiment with regard to polynucleotides, example 13 of Felgner teaches that such associations might favorably be simply a complex.

The Office appears to take the view that a reason for applying the Kirby method to ensure intravesicular incorporation of the expression system is found in either Weiner or Liu. Weiner,

however, for the most part, merely teaches the administration of naked DNA. The only mention of liposomes noted by the Office is in column 20 at lines 37-49, which states that in some embodiments "the genetic construct is administered as part of a liposome complex with a response enhancing agent." This falls far short of suggesting that the genetic construct should be incorporated into the intravesicular space of a liposome. Indeed, Weiner, like Felgner, appears to teach away from entrapment into the intravesicular space by suggesting merely a complex.

Similarly, Liu is directed to the administration of naked DNA. This would hardly suggest to the reader that steps should be taken to ensure that the expression system resides in the intravesicular space of a liposome.

Taken as a whole, the cited art fails to provide to the practitioner any reason to apply the methods of Kirby to obtain liposomes that entrap expression systems for immunogens in the intravesicular space, as opposed to simply administering the expression systems, along with a cationic lipid or simply complexed to liposomes.

Accordingly, applicants believe no *prima facie* case has been made by the Office.

Unexpected Results

Even if a *prima facie* case has been made, applicants point to the surprising results set forth in the specification which show that the invention compositions perform consistently and dramatically better than either naked DNA or complexed DNA in eliciting immune responses. These results are surprising and unexpected as was pointed out by applicants in the parent application, US 09/254,695. In response, the Office had stated that the surprising results were not commensurate with the scope of the claimed invention because a significant difference was noted at

only one of the two experimental conditions (10 μg versus 1 μg DNA) and only at 21 days and not 28 days. Claim 1 has been amended to require that the polynucleotide be administered in an amount sufficient to elicit an immune response. It is not entirely clear from Table 5 whether a dilution factor of only 2.2 represents any response at all, since naked DNA appears to register the same dilution regardless of amount and the same is true of the immunization with non-cationic liposomes (Test Reports (e) and (f) in Table 5).

In any event, the criteria cited by the Office in the rebuttal offered in the Office action mailed 6 January 2003 in US 09/254,695 does not really address scope. It would be expected that an immune response would vary with dosage and that it would vary with the length of time between administration and measurement. This is not a matter of scope, but a matter of timing and dosaging for ascertaining the appropriate comparisons. It appears that 1 μg of injected DNA is simply too low to elicit much of an immune response with any formulation. However, raising the dosage to 10 μg in the case of the entrapped DNA entrapped in a cationic liposome as required by the claims dramatically increases the ability of the composition to elicit an immune response after 21 days, whereas raising the dosage to 10 μg has no effect with regard to any of complexed polynucleotide, polynucleotide in non-cationic liposomes and naked DNA. This simply means that at comparable dosages, the DNA entrapped in cationic liposomes is much more effective.

Similarly at 28 days, there is no effect of increasing the dosage of naked DNA, and only minimal effects are observed with complexed DNA or DNA associated with neutral liposomes, but there is a dramatic increase for 10 μg over the results with 1 μg in the case of the invention compositions.

Applicants believe that the proper comparison is between the invention compositions and the compositions suggested by the prior art under the same conditions. When the compositions of the invention and those of the prior art are compared under the very same conditions, the invention conditions dramatically out-perform those of the prior art. The fact that there are some conditions where neither the prior art compositions nor those of the invention are sufficiently potent to elicit an immune response, does not undermine the results obtained under conditions where the invention compositions succeed even though the prior art compositions are too weak.

Further, as discussed at the interview, the results are perhaps more dramatic than they appear at first glance, since the values shown in the tables are logarithms to the base 10 of the dilution factors required in the assay. Thus, in Table 5, 28 days after immunization with 10 μg of plasmid DNA expressing the hepatitis B surface antigen (page 23, lines 2-3), the dilution required for this plasmid entrapped in cationic liposomes (test report b) is $10^{4.0 \pm 0.2}$ whereas that for complexed plasmid (test report d) is only $10^{2.8 \pm 0.2}$. This represents not a 30% increase, but more than a 10-fold difference. Similarly, in the 21 day column, the result for entrapped plasmid is $10^{3.2}$ whereas that for complexed plasmid is only $10^{2.2}$, again a 10-fold difference. (Compared to naked DNA, the results are even more dramatic representing almost a 100-fold difference for entrapped DNA as compared to naked.)

The foregoing unexpected results for humoral responses are reflected in cellular responses as well, with respect to subcutaneous and intramuscular administration, to which the claims are limited. For example, in Figure 7, with subcutaneous injection, the concentration of IL4 generated by the entrapped plasmid is about 4-fold higher than that generated by naked DNA. Similar results

are obtained for IFNγ in Figure 8. (It is noted that the designation of the figures is reversed from that set forth in the text on page 31, beginning at line 9.)

Thus these results demonstrate that both Th1 and Th2 responses are obtained.

Respectfully, no one would have predicted this effect based on the art cited by the Office.

If there is some reason to think that similar disparities would not be shown with regard to other immunogens, applicants believe it is the responsibility of the Office to explain why this would be the case. It is certainly true that some immunogens are more immunogenic than others, but there is no reason to think that the same differences between delivery systems would not be found.

Applicants agree that unexpected results in support of patentability should reflect the scope of the invention as claimed. It is respectfully submitted that they do. Applicants have illustrated a typical system characteristic of the invention and compared it to systems typical of the prior art and the Office has provided no reason that different immunogens would result in different results.

Summary

In summary, because there is no suggestion in the art that inclusion of expression systems for immunogens should be confined to the intravesicular space of liposomes as opposed to associated with cationic lipids generally, there is no reason to combine Felgner, Liu or Weiner with a disclosure (Kirby) of a method for incorporation into the intravesicular space. Even the *KSR* decision acknowledges that there must be some reason for the artisan to combine teachings in the art in order to arrive at the invention. Thus, there is no *prima facie* case.

Even if a *prima case* were made out, the unexpected results in the specification support the patentability of the invention as claimed.

Claim 7

The remaining claim 7 was rejected as unpatentable over the same combination of Felgner, Kirby and Weiner in further review of Collins (US Patent 5,567,433).

First, claim 7 is patentable for the same reasons as set forth above with regard to claim 6 from which it depends. The Office cites Collins only for disclosing microfluidization.

However, a careful reading of Collins shows that the microfluidization step is not suggested subsequent to loading the liposomes with the biologically active agent but rather as a means to do so. As noted in column 5, beginning at line 9:

> The hydrated lipid: material mixture is then optionally microfluidized in order to fully mix lipid and solute and *provide encapsulation of the solute*. (Emphasis added).

Thus, it is clear from Collins that microfluidization is performed in the loading step, not afterward as is required by claim 7. Accordingly, the disclosure of Collins does not give applicants a reason to microfluidize the already loaded liposomes.

Conclusion

With regard to all pending claims, other than claim 7, the combination of documents cited does not provide a reason for applicants to use the method of Kirby in encapsulating expression systems for immunogens. Rather, the Felgner document teaches that it does not matter how the expression system (among other biologically active agents) is associated with a cationic lipid. Felgner teaches that it is a matter of indifference rather the expression system for an immunogen is simply complexed to a cationic lipid, associated with a vehicle containing a cationic lipid, or encapsulated within said vehicle. In any event, it is not clear from Felgner that "encapsulated"

means contained within the intravesicular space, as this term is not defined. It could very well simply mean only associated therewith. The teachings of Weiner and of Liu actually discourage the application of the Kirby method to encapsulating expression systems, since these documents teach that it is unnecessary to encapsulate expression systems at all in order to elicit an immune response.

Even if a *prima facie* case had been made out, the unexpected results shown in the specification provide support for patentability. There is no hint that encapsulation in the intravesicular space would yield greatly improved results, in eliciting both humoral and cellular responses as compared to simple complexes, neutral liposomes or naked DNA. The scope of the unexpected results is commensurate with the scope of the claims since there is no evidence of record that immunogens other than that tested would behave in a different way as compared to the illustrative immunogen.

Finally, claim 7 is not suggested by the cited documents in combination with Collins, because Collins suggests microfluidization as a loading technique, whereas microfluidization as required by claim 7 is conducted after the liposomes are already loaded.

For these reasons, applicants respectfully submit that all pending claims, claims 1, 6-8, 11-14, 16-20, 28-31, 34-37 and 39, are in a position for allowance and passage of these claims to issue is respectfully requested.

Again, applicants greatly appreciate the helpful interview with Examiner Schnizer. If any issues remain that may be resolved by telephone, a call to the undersigned at the indicated number is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of

such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 429022000620.

Respectfully submitted,

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